Polypharmacology in Drug Discovery: A Review from Systems Pharmacology Perspective

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Abstract: *Background:* The complexity of diseases has led to recent interest in polypharmacology, which suggests that many effective drugs specially modulate multiple targets. Drugs with multiple targets can provide a superior therapeutic effect and decrease in side effect profile compared to ligands with single target, especially in the treatment of complex diseases, such as tumors, nervous system diseases and inflammatory diseases. The network-based polypharmacology holds the promise of expanding the opportunity for novel targets and drug identification. However, it faces considerable challenges to how multi-target drugs can be rationally designed from the network pharmacology perspective, particularly for combinations of targets that are structurally divergent. *Methods:* In this review, we focus on the pharmacological properties of current polypharmacology, discuss potential novel drug indication arising from drug repurposing, and introduce approaches to the rational design of multi-target drugs. *Results:* As a result, we highlighted the features of polypharmacology. Also, we have presented some computational methods to predict the potential novel multi-target drugs with lower toxicity and higher efficacy. Moreover, network analysis might play important role in repositioning drugs that modulate targets involved in different pathologies. *Conclusion:* This perspective aims to provide a global view on polypharmacology, which is the foundation of the next paradigm in drug discovery.

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Keywords: Polypharmacology, multi-target drugs, drug combination, drug repurposing, network pharmacology, drug design.

1. INTRODUCTION

Medical industries aims at identifying drug candidates with efficacious and well-tolerated, safe medicines to ensure them successfully completing clinical trials, reaching the market [1]. Such drug candidates require a favorable pharmacological and physico-chemical properties. In the past decades, targeting a single kinase has been proven successful in the treatment of oncogenesis, for example, drugs that inhibit BCR-ABL, as well as members of the epidermal growth factor receptors (EGFR) and rapidly accelerated fibrosarcoma (RAF) class of proteins [2-4]. However, despite considerable progress in genome- and proteome-based high-throughput screening methods and rational drug design, the number of successful drugs of novel single-target did not increase appreciably [5, 6]. This is due to that single-target drugs tend to exhibit low clinical efficacy typically the attrition rate as high as 90% at the late stage of clinical trials [7, 8]. Additionally, many diseases with unmet therapeutic needs are in essence complex and multifactorial, the underestimation of their complexity might be the reason behind their medical failures [9]. In fact, it has been appreciated that many effective drugs act on multiple targets rather than single target, the concept of polypharmacology [10, 11]. Polypharmacology currently encompasses both multiple drugs that act independently on different targets, and a single drug binding to multiple targets within a biological network, as opposed to the concept of "one gene, one drug, one disease" [12, 13]. In recent years, the efficacy of multi-target therapy is supported by observations concerning the robustness and resilience of complex biological systems. For example, most approved kinase drugs potently inhibit multiple targets, and they are attractive therapeutic agents for numerous disorders ranging from neurology to cancer [14-17].

More importantly, polypharmacology is referred to the specific binding of a drug to two or more molecular targets in networks, the robustness and redundancy of biological systems. In a biological network, polypharmacological drugs are involved in two different ways: a single drug acting on multiple targets of a unique disease pathway and a single drug acting on multiple targets associated to multiple disease pathways [18]. Thus, in principle, multi-target therapeutics can achieve greater efficacy and be less vulnerable to drug resistance by impacting multiple nodes at the system level [19]. Indeed, when faced with complex diseases, networks of drug-target, target-disease networks may be able to resort the system robustness to ensure the effective treatment. As a conclusion, polypharmacology focuses on searching for multi-target drugs to perturb multiple disease-associated networks rather than designing selective ligands to target individual proteins, which brings a holistic view into new drug development.

The efficacy and toxicity of drugs, whether designed as single- or multi-target therapeutics, result from complex interactions between pharmacodynamics, pharmacokinetic, genetic, epigenetic, and environmental factors. Meanwhile, drug resistance is usually triggered by the appearance of one or more mutations in the genetic encoding for drug target proteins [20, 21]. With the help of poly-pharmacological drugs, the probability of a cell developing resistance simultaneously to multi-target drugs acting on unrelated proteins is statistically lower than the probability of resistance developing against single-target drugs [22]. Moreover, drugs acted on multiple targets can decrease drug doses, so that less efficacious and slightly more toxic compounds can be used safely. In addition, compounds with favorable polypharmacology that engage multiple targets and do not hit on off-targets can have excellent therapeutic efficacy with reduced toxicity and resistance [7, 14, 23, 24]. Consequently, the identification of bioactive small molecules that specifically multi-target proteins with good therapeutic effect and less toxicity are at the heart of chemical biology research. As mentioned above, the use of polypharmacology may offer an excellent alternative to establish biological methodology or provide entirely different opportunity to gain new insight for the novel drug development.

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In recent decades, much progress was made in establishing methods for the identification of the cellular targets of small molecules. One of the main characteristics of target-based approaches is the focus on drug targets. However, it is not a generally applicable methodology that can successfully be applied in the majority of the cases, so screening the polypharmacological drugs with maximal efficacy and minimal toxicity is the rational discovery. To date, only approximately 2% of all the predicted proteins have been targeted with drug molecules and the estimated fraction of potential "druggable" proteins is approximately 15% [25]. Recently *in silico* systematic prediction methods have been the popular strategies to increase the efficiency and safety of drugs. For example, Zoltan *et al.* proposed an approach related complex drug-protein interaction profiles on the basis of the relationship between 177 major effect categories and 1200 FDA-approved, finally, they predicted uncovered effect profiles of drugs in a systematic manner [26]. Also, Campillos used phenotypic side-effect similarities to infer whether two drugs share a target, identifying 13 implied drug-target relations out of 20 predictions by *in vitro* binding assays [27].

Nevertheless, polypharmacology approaches could provide drug candidates with a superior efficacy profile, it is important to note that we are still facing several challenges. The problem is, how do we rationally approach targeting multiple protein targets? Which targets are most likely to be modulated by a single drug? Or, conversely, which drugs are most amenable to multi-targeted drug design? And how do we avoid the toxicity resulting from polypharmacology? Rational design of multi-target compounds is still in its infancy, so it surely will need further novel methodological development. In this review, we first discuss the pharmacological properties of polypharmacology, and then reviewed the computational methods for establishing pharmacological relationships between proteins, and how these values can be used to construct interaction networks that can guide the design of multi-targeted drugs. The framework of the review was shown in Fig. **1**.

2. MULTI-TARGET DRUGS AND COMBINATON THERAPY

For many years, the paradigm of drug discovery was to develop highly selective ligands that interact with individual target proteins [7]. The genome projects has offered profound drug-development strategies by the wealth of potential targets. However, despite the considerable drug-development efforts undertaken, the number of successful drugs and novel targets did not increase appreciably during the past decade. Agents that act on one target only might not modulate complex systems in the desired ways even if they can change their targets immediately. Furthermore, single-target therapeutic agents could induce side effects and tissue toxicity, resulting

in reduced efficacy, drug resistance, and a generally decreased quality of life for patients [22]. These considerations are independent for the drug discovery of whether or not the pharmacological agent inhibits or activates its targets. Therefore, the classical 'one drug for one target for one disease' perspective is an oversimplification challenged by a growing body of evidence showing that there are polypharmacological drugs generally enjoyed by more clinical success than highly selective alternatives [7, 23, 28, 29]. The trends of drug development was shown in Figure 1, which illustrated the drug design process from the single-target to multi-target with the aid of computational screening and *in vitro* screening. In this part, we divide the polypharmacology into two different forms: single drugs of multiple targets and the combination therapy.

The first form of polypharmacology is combination therapy, which employs different drugs with different mechanisms of action to treat diseases [30]. The combinatorial drugs currently are employed for rational design, and their increased efficacy justifies in vitro discovery efforts for identifying novel multi-target mechanisms [31]. The drug combination therapy represents the most simple and immediate way to regulate perturbation of the pathogenic cascade. This approach is a holistic paradigm, which has already been proven effective in combating complex diseases, including tumors, HIV infections and hypertension [9]. For example, preliminary clinical studies suggest that some of drug combinations include kinase, heat shock protein 90 (Hsp90), and farnesyltransferase inhibitors exhibit promising clinical efficacy in cancer clinical trials [32]. That is because that molecules targeting multiple independent targets on different mutation sites may achieve the synthesis therapeutic effect by preventing drug resistance and reducing side effects. In addition, by aiming for a weak perturbation of the biological network, lower doses of each compound can be used, resulting in better therapeutic selectivity in many important areas [33]. However, combining several drugs in a single pill is not always an easy task. Further difficulties arise when specific combinations are used for clinical development [34]. Another drawback of the combination therapy is the drug-drug interaction. Drug interactions can occur at all levels in the body and can be attributed to multiple mechanisms, thus resulting in variability in drug exposure. Moreover, failure to identify drug interactions can thus lead to overdosing or under treatment, with severe clinical consequences. Given this, polypharmacology has recently been shifted towards the multi-target approach.

Multi-target drug therapy is the other form of polypharmacology. The ability of drugs to act at multiple targets have been known to medicinal scientists for decades [35]. The promiscuous drugs can interact with multiple targets that additively overcome the robust nature of biological networks [7, 24]. Multi-target drugs have
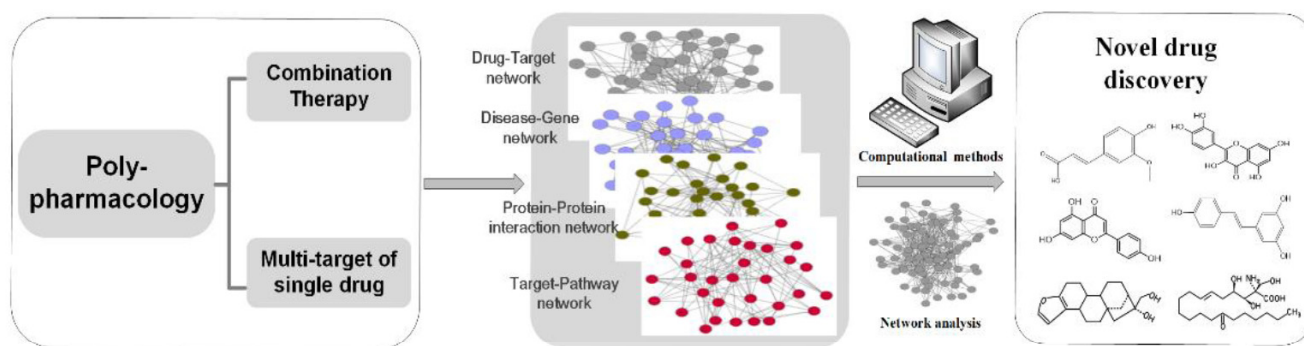
Fig. (1). Polyphamacology serves an integral role in systems approaches to drug discovery. The two forms of polypharmacology are combination therapy and multi-target. Network pharmacology provides a global template for novel drug prediction. In networks, nodes represent genes, diseases or small molecules and edges connecting these nodes represent the physical interactions such as co-expression or some other shared properties linking the nodes. For example, drug-target network and protein-protein interaction network decipher the action mechanisms of medicines. Therefore, network approaches facilitate efforts in drug discovery and systems pharmacology. And the details of these various network-based systems approaches are outlined in the text.

therapeutic advantages over single-target drugs because they can show either additive or synergistic effects. In fact, the majority of approved drugs modulate multiple targets to achieve the desired treatment [23, 28]. For example, many multi-targeted tyrosine kinase inhibitors (TKIs) such as imatinib (1) [36, 37], nilotinib (2) [36, 38], and vandetanib (3) [39] are approved for clinical use to treat solid tumors in 2010 [37, 38, 40]. (The chemical structure of representative drugs are shown in Figure 2.) Psychiatric drugs such as clozapine has good efficacy by targeting on several well-defined proteins: a number of serotonin (5-HT$_{2A}$, 5-HT$_{2C}$, 5-HT$_6$, 5-HT$_7$), dopamine (D4), adrenergic (α1-and α2-subtypes) and other biogenic amine receptors [41]. Several non-steroidal anti-inflammatory drugs (NSAIDs), such as salicylate (4), metformin (5) or imatinib (1) serve as highly effective drugs by affecting many targets simultaneously. Furthermore, compounds with favorable multiple targets in a rational fashion can have excellent therapeutic efficacy with reduced toxicity and resistance. That is because that a drug is unlikely to bind to a variety of different targets with equally high affinity. However, the low-affinity of rug binders is not a disadvantage. For example, multi-target noncompetitive NMDA receptor antagonists (agents used to treat Alzheimer's disease) exert low-affinity, and multi-target drugs are likely to have a reduced side effects than single-target drugs with high affinity [42, 43]. In addition, the proteins in the signaling and transcriptional networks are always interacting with their partners with low affinity [43]. Thus, it is suggested that multi-target attacks block an increased number of individual interactions (network links) than a single knockout. The simulation explains the higher efficiency might be that, multiple attacks can be more efficient than a single attack even if the number of affected interactions is the same [44]. Therefore, even most multi-target drugs are weak linkers, which might be sufficient to achieve a significant modification and desired therapeutic effect.

In contrast, multi-target drugs with multiple biological properties could have inherent advantages over combination therapies, this is mainly due to different bioavailability, pharmacokinetics (PKs) and metabolisms [9, 30]. Moreover, multi-target drugs offer the chance to overcome the intrinsic conflict of combination therapies, where effectiveness deriving from hitting the disease network on multiple fronts. The successes of multi-target drugs have contributed significantly to the increasing attractiveness of polypharmacology in a single molecule, therefore, in the future, the rational design of single-molecule drugs with a desired multi-target profile may offer an appealing and cost-effective alternative to drug development. On the other hand, the combination therapy of polypharmacology also play an important role in the drug design. The success stories of both multi-target drugs and combination therapies suggest that both approaches are potentially able to promote the new drug development or drug combinations with improved safety
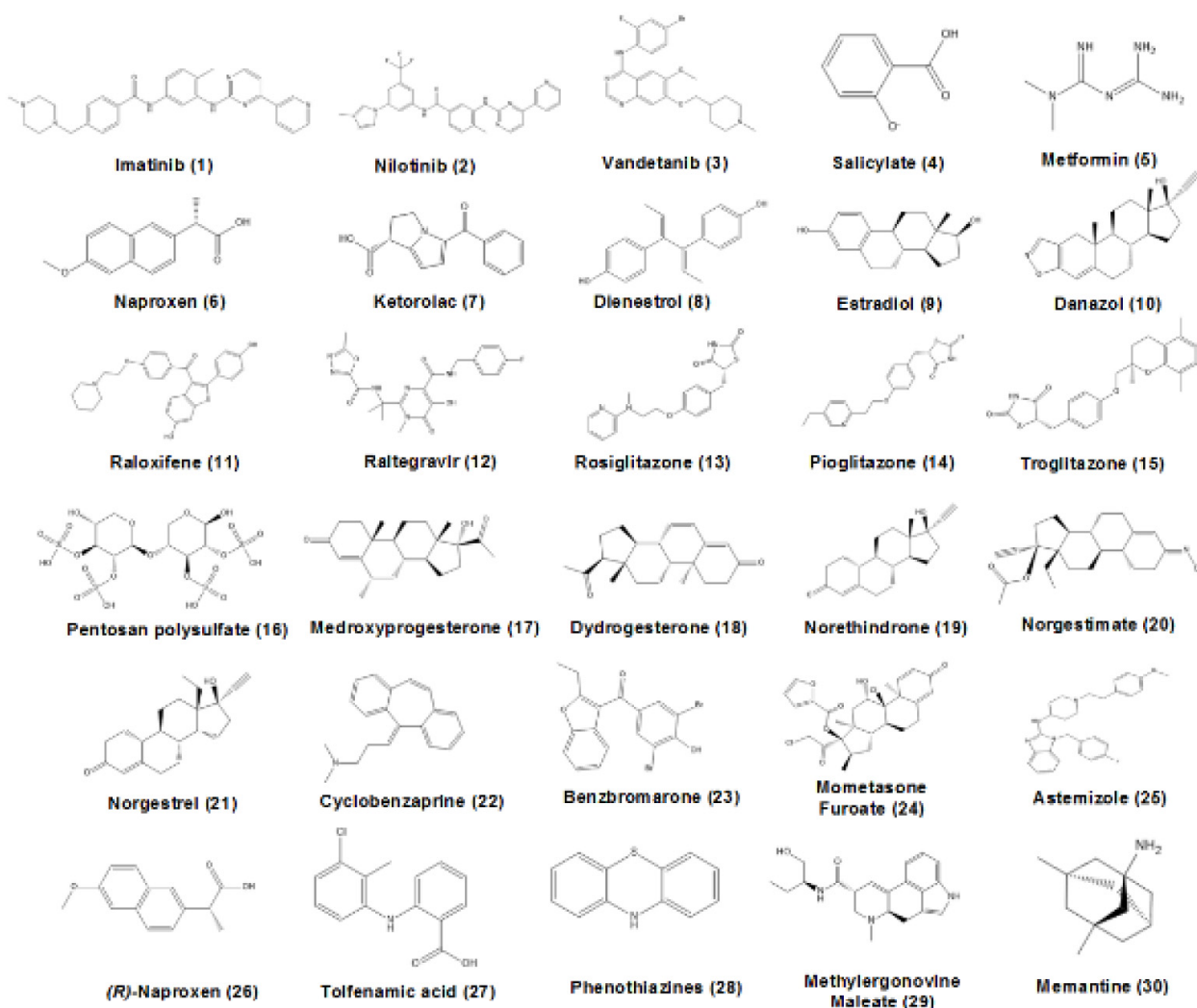

Fig. (2). Chemical structures of representative drugs and pharmaceutically relevant compounds discussed in the review.

and efficacy profiles. Hence, we proposed that systematic drug-design strategies should be directed against multiple targets, which might promote the development of more-efficient molecules than the currently favored single-target drugs.

3. DRUG REPURPOSING

An important implication of polypharmacology is drug repurposing, which seeks to discover new clinical application of existing drugs used in the treatment of a given pathology [45-47]. The already approved drugs are more probably to be repurposed for other neglected and rare diseases, thus extending the chemical libraries other than known drug [48]. The major advantage of drug repurposing approach is that the pharmacology and toxicity profiles of drugs are already well known in the preclinical and Phase I studies. Thus, these drugs could be rapidly translated into Phase II and III clinical studies and the associated cost could be significantly reduced [49]. Furthermore, the medical companies are attracted by drug repurposing with respect to efficacy (e.g. for the novel indication), and sometimes for safety as well (e.g. when doses higher than the approved ones are needed) [50]. Therefore, the interest in drug repurposing seeks to balance both profit and the service drive, which could save time and economical cost in the drug discovery process. Currently, drugs for repurposing today would involve in a new use obtained in and beyond clinical trials. For example, two nonsteroidal anti-inflammatory drugs (NSAIDs), the *(R)*-enantiomers of naproxen (6) and ketorolac (7), have exerted specificity for inhibiting Rho family GTPases, in particular Rac and Cdc42. These two drugs are potential candidates as adjuvant therapy to prevent ovarian tumor growth and dissemination during postsurgical recovery [51]. Wang *et al.* analyzed 61 approved drugs targeting on 16 cancer genes from the Therapeutic Target Database (TTD), inferring 11 approved drugs that are not relevant to cancer may be repositioned as anticancer drugs by combining the medical genetic information of the targets [52]. These drugs was listed in Table **1**. Among them, dienestrol (8) and estradiol (9), two estrogen receptor alpha (ESR1) agonists, have been clinically used to treat atrophic vaginitis and serve as hormone replacement, respectively. Indeed, the breast cancer-inducing property of dienestrol and estradiol were

recorded in Drugs.com (http: //www.drugs.com/), and the anti-breast cancer activity of danazol (10) and raloxifene (11) have been validated by experiments (Table **1**) [53, 54]. In addition, several molecules related to drug repurposing are available in Table 2 by US National Institutes of Health (NIH) on April 21 2011 with pharmaceutical industry leadership. These drugs have been proven safe in clinical trials [55-58]. For example, raltegravir (12) is HIV-1 integrase inhibitor approved by the FDA in 2006, Rob Hromas, MD (UNMCC) and clinical scientists at UNM identified it as a potential drug for adjuvant therapy in cancer [59, 60]. Phenothiazines used to bind to versatile targets and exhibit several desirable therapeutic effect. Phenothiazine derivatives have been used as antimalarials (late 19th century), antihelminthics (mid-20th century), antihistaminics (1940s), sedatives, and antipsychotics (1950s). Nowadays, scientists suggest that phenothiazines and their derivatives are potential drugs for the treatment of Parkinson's and Alzheimer's diseases, and as antibacterial and antifungal compounds [61]. However, these drugs targeted novel proteins are currently under investigation to determine the mechanism of action. Besides, how to discern the various functions of already approved drugs and target profiles is an unsolved problem.

Recent developments have opened the door to using drug repurposing approaches that rely on both empirical data and computational models. The inverse genomic signature approach is based on the premise that an effective drug targets on a gene expression profile that is inversely correlated to the host signature associated with the disease. The approach integrates the complexity of the genome-wide response of the host to both the disease and the treatment, it is rooted in scalar theory [68-70]. Importantly, there has already been successful application for several disease indications [71]. On the basis of the approach, a public database Connectivity Map (cMap) (http: //www.broadinstitute.org/cmap/; version 2) that covers over 6000 transcriptome profiles established downstream of treatment of human cell lines with over 1300 compounds. Then, Lamb *et al.* modified the computational approach, and they used it to compare a profile of 164 known drug compounds in cMap to an inflammatory bowel disease (IBD) specific gene expression signature derived from 176 datasets available in Gene Expression Omnibus (GEO)

Table 1.   Predicted new indications for 11 approved drugs.

No.	Drugs	Current drug indication	Predicted New indication	Refs.
1	Danazol (10)	Endometriosis	Breast cancer	[62]
2	Raloxifene (11)	Osteoporosis in postmenopausal women	Breast cancer	[63]
3	Rosiglitazone (13)	Diabetes mellitus	Colorectal cancer	[64]
4	Pioglitazone (14)	Diabetes mellitus	Colorectal cancer	[65]
5	Troglitazone (15)	Diabetes mellitus	Colorectal cancer	[66]
6	Pentosan polysulfate (16)	Interstitial cystitis/painful bladder syndrome	Breast cancer	[67]
7	Medroxyprogesterone (17)	Hormonal contraceptives	Endometrial cancer	ClinicalTrials.gov
8	Dydrogesterone (18)	Menstrual disorders	Endometrial cancer	n.a.
9	Norethindrone (19)	Oral contraceptive	Endometrial cancer	n.a.
10	Norgestimate (20)	Hormonal contraceptives	Endometrial cancer	n.a.
11	Norgestrel (21)	Hormonal contraceptives	Endometrial cancer	n.a.

Abbreviation: n.a., not available; Drugs.com (http: //www.drugs.com/); ClinicalTrials.gov (http: //clinicaltrials.gov/).

Table 2. Summary of drug repurposing at UNM.

No.	Drugs	Current targets/indication	New targets/diseases
1	Raltegravir (12)	HIV-1 integrase; antiviral for treatment of HIV-infected patients	Metnase; adjuvant therapy in cancer
2	Cyclobenzaprine (22)	Not described; skeletal muscle relaxant	Mono-amine transporters and serotonin receptors; may cause serotonin syndrome
3	Benzbromarone (23)	Xanthine oxidase; uricosuric for treatment of gout	Quorum sensing signaling pathway; anti-bacteria
4	Mometasone Furoate (24)	Glucocorticoid receptors; for treatment of seasonal allergy	P-glycoprotein; adjuvant therapy in cancer
5	Astemizole (25)	Histamine H1 receptors; antihistamine for treatment of seasonal allergy	Inducer of autophagy; as adjuvant therapy in prostate cancer
6	(R)-Naproxen (26)	Cyclooxygenases; nonsteroidal anti-inflammatory drug for short-term treatment of pain	RAC and CDC42GTPases; as adjuvant therapy in cancer
7	Ketorolac (7)	Cyclooxygenases; nonsteroidal anti-inflammatory drug for short-term treatment of pain	RAC and CDC42GTPases; as adjuvant therapy in cancer
8	Tolfenamic acid (27)	Cyclooxygenases; nonsteroidal anti-inflammatory drug for short-term treatment of pain	Inhibitor of hantavirus/DAF binding; antiviral against Sin Nombre virus
9	Phenothiazines (28)	Prototype for neuroleptic drugs; antipsychotics for the management of schizophrenia	VLA-4; anti-adhesion inhibitors against inflammation and cancer
10	Methylergonovine maleate (29)	Oxytocic; for treatment of postpartum uterine hemorrhage	Bcl-2 family proteins; anti-apoptotic as adjuvant therapy in cancer
11	Beta-adrenergic receptor drugs	Beta-2 adrenergic receptor agonists are used for the therapeutic management of asthma	Noncannonical G-protein coupled receptor ligands

[72, 73]. As a result, two strongest anti-correlated drugs-prednisolone and topiramate were identified to treat the IBD disease. In addition, Josset *et al.* used the host transcriptional response to influenza virus to identify potential therapeutics against influenza in the cMap database [74]. Finally, eight candidate drugs were identified and approved for novel indications, six of these drugs inhibited viral growth in *in vitro* assays. And most importantly, five out of the eight also inhibited the growth of the pandemic 2009 H1N1 influenza virus. In conclusion, these new computational approaches have the advantage to greatly reduce both the time and cost for the novel drug discovery.

Although successful drug repositioning campaigns currently become a new approach for the support of drug discovery and development, as well as clinical trial, there are two limited conditions that deserve attentions, (i) the drug acts at the same single target but with different effect that attribute to the physical site of biological action; (ii) if the "old" function of the drug and the "new" one may hit different biochemical targets, it is extremely unlikely that the repurposed drugs have been preoptimized for the new effect [43]. These problems lead to the familiar situation, where the repurposed drug, even if first-in class to the clinic, that would eventually be rendered invalid instead of a subsequent drug that would be even better. Therefore, medicinal chemists have sought to incorporate new tools and approaches to search for new and better drugs by drug repurposing.

4. NETWORK PHARMACOLOGY

Network-based approach has becoming more and more powerful tool for drug-target analysis. Various networks help to identify the function of novel proteins and thus increases the number of potential targets [75, 76]. For the analysis of multi-target drugs that affect specific disease models (e.g. anti-hypertensive, anti-psychotic and anti-diabetic drugs), specific network models are needed. The analysis of drug-target network might decipher the action mechanisms of medicines and discover the most potential "follow-on" drugs, with the bridge connection of targets [77]. In addition to drug-target network, disease-gene network, protein-protein interactions and multi-pathway networks also can be subjected to a similar analysis [78]. In these network models, each node represents a protein/gene/disease, and each edge corresponds to an interaction between two elements. The interactions of these nodes represent the physical interactions, genetic regulatory interactions and higher order relationships such as coexpression or some other shared properties. In networks, most nodes have only a few interactions, however, the proteins coexist with a few highly connected nodes, hold the whole network together. In this article we review recent advances in the field of network pharmacology. Empirically modelling cellular networks have provided the necessary support for understanding the functional, logical and dynamical aspects of cellular systems. Importantly, we discuss how genes and their products interacting with each other form complex networks within cells and the possibility that phenotypes result from perturbations of the properties of networks.

4.1. Drug-Target Network

Many successful drugs bind to and modulate multiple targets *in vivo,* successfully navigating drug-target network might be fruitful to discover new drugs or novel targets for existing drugs. Additionally, identification of conserved interaction patterns of drug-target network with distantly related proteins is crucial for target identification in polypharmacology [79]. For example, Mestres *et al.* analyzed a drug-target network consisting of 4767 unique interactions and 802 drugs, leading to a conclusion that a drug interacts with 6

targets on average [80]. Yildirim *et al.* constructed a network of FDA approved drugs and drug targets, revealing a rich network of polypharmacology interactions [81].

By now, a variety of computational methods have been proposed to analyze and detect new drug-target interactions. For example, the Ligand-based approach like QSAR (Quantitative Structure Activity Relationship) uses machine learning methods to predict protein-ligand interaction by comparing a new ligand to the known ligands of a target protein [82, 83]. QSAR studies are undoubtedly of great importance to select the most promising compounds in modern chemistry and biochemistry. However, if the number of known ligands for a target protein of interest is insufficient, this approach couldn't predict the interactions effectively. In addition, target-based approach or docking simulation relies on the 3D structure of proteins to predict protein ligand interaction and it can't be applied to proteins with unknown 3D structure [84-86]. These methods are limited for membrane proteins such as Ion channels and G-Protein Coupled Receptors (GPCRs) due to the complexity of determining 3D structures of most of these proteins. Recently, the importance of chemogenomic approaches in the domain of protein ligand interaction prediction has grown fast [87-89]. These methods integrate both genomic spaces of target proteins and chemical space of compounds to predict new drug-target pairs. Also, inverse docking, one of the typical structure-based methods, is widely used to predict protein targets of small molecules [90-92]. Takigawa *et al.* developed a fast, scalable algorithm to capture significant paired patterns of subgraph-subsequence from drug-target interactions of approved drugs, dividing drug-target interactions into clusters. These exclusive clusters are naturally found by highlighting significant substructure pairs in drug-target interactions rather than using either drug or target information only, which confirm the effectiveness for interpreting polypharmacology in drug-target network [93].

Another success in predicting drug−target interactions has been developed by using drug side-effect similarity [27], machine learning approaches [93, 95, 96], and complex network theory. Violeta *et al.* proposed the Gaussian ensemble screening (GES) "computational polypharmacology fingerprint" (CPF), the first target fingerprint to encode drug promiscuity information, which can successfully describe drug-target relationships and can serve as a novel method for proposing new targets for preclinical compounds and clinical drug candidates [97].

In a network, if individual targets are redundant, then the drugs could interact with multiple targets to exert better therapeutic effects [7, 98]. Recently, Huang *et al.* published a comprehensive review on multi-target therapies of herbal medicine in depression [99]. They reported that some antidepressant drugs bind to more than 20 targets, indicating potential synergistic mechanism in herbal mixture for treating the disease. Drug targets tend to have more interactions than average proteins to a statistically significant degree [98]. Drugs with more proteins tend to be valid clinically and are labeled as 'follow-on' drugs [100]. Moreover, it is sufficient that these multi-target drugs affect their targets with the presumed low-affinity interactions to affect the complex equilibrium of whole biological networks [99]. Thus, multi-target drugs can increase the number of weak links in cellular networks and stabilize these networks in addition to having multiple effects. All these information mentioned above illustrates the importance of drug-target network in the promising polypharmarcology.

4.2. Disease-Gene Network

The target-disease network is a bipartite graph between diseases and their therapeutic genes, where nodes represent diseases and their therapeutic genes, and the edges represent the interactions of diseases and genes. This network is essential to understand the similarity and difference in treating different diseases, moreover, it helps us to explore the potential therapeutic effects for drug com-

pounds of the known targets. In the previous study, we have built the disease-gene networks between the ingredients of various Chinese medicines and their related diseases [102-104]. For example, Liu *et al.* built the disease-gene networks to illustrate the mechanism of licorice as cough reliever, anti-inflammatory, anti-anabrosis, immunomodulatory, anti-platelet, antiviral (hepatitis) and detoxifying agent. Based on these different protein-disease networks, novel therapeutic targets such as 5-HT$_{2A}$ (5-hydroxytryptamine 2A receptor) and AKR1B1 (aldose reductase) related to diabetic complications and MAOB (monoamine oxidase type B), D2 and D3 dopaminergic receptors and MAPK10 (mitogen-activated protein kinase 10) involved in neurological disease were identified in licorice [104].

A disease-gene network linked by known disorder-gene associations offers a platform to explore the common genetic origin of many diseases. Genes associated with similar disorders show higher likelihood of physical interactions and higher expression profiling similarity, indicating the existence of disorder-specific functional modules. In fact, the majority of nodes have few links while other nodes, and the nodes have a much higher degree of linkages called hub proteins. Hub proteins have been considered as more essential and more abundant, and they show a greater diversity of phenotypes in knockouts compared to nonhub proteins [105-109]. Goh *et al.* found that the vast majority of disease genes are nonessential, exhibiting no function to encode hub proteins, and the expression pattern of these genes are localized in the functional periphery of the network [110]. Indeed, the human disease-gene network reveals not only drugs targets on multiple proteins, but also drug targets are always involved in multiple disease. To assess the relationships between the genes and diseases, the functional human gene networks integrating information on genes and the functional relationships between genes were constructed by Franke *et al.* Their study indicated that it is feasible to use gene networks to prioritize positional candidate genes in various disorders with multiple associated genes [111].

4.3. Protein-Protein Interaction Network

In recent decades, significant steps have been taken toward the generation of comprehensive protein-protein interaction network maps. In protein-protein interaction networks, nodes represent proteins and edges represent a physical interaction between two proteins [112]. These networks provide insight into the origins of overall cellular behaviors and evolutionary design principles, as well as fields of concerning specific cell biological processes or diseases. For in lung squamous cancer tissues, the up-regulated genes of differentially expressed genes have significantly higher connectivity in the PPI network [113]. Similarly, Jonsson and Bates reported that cancer-related proteins have approximately twice the interaction partners than that of proteins unrelated to cancer [114]. Another observation is that disease gene are more likely to encode hubs in the PPI network than nondisease genes [110]. More importantly, the interacting pairs are experimentally confirmed in Interacting Proteins Database [115]. Oti *et al.* used the protein-protein network to predict disease genes for genetically heterogeneous hereditary diseases [116]. The research was based on the assumption that if disease proteins were located within other loci associated with that same disease interacted, they were considered as candidate disease genes. To conclude, the interaction of protein−protein interactions can promote the positional candidate disease genes.

4.4. Multi-Pathways

To globally reflect the interactions of targets and disease-related biological pathways, the target-pathway interaction network is used to interpret the relationship of the targets and pathways. Mapping to a drug-target network allows us to prioritize new selective compounds, while mapping to other biological networks enable us to

observe interesting target pairs and their associated compounds in the context of biological systems [117]. Among all the pathway-networks, the cell signaling pathways are the most important and usually woven together, signaling from different sources of stimuli would activate the same downstream target, and induce the same cell function. For example, Gong *et al.* presented the cases of alternative pathways resulted from the available experimental data, and revealed that alternative signal pathways could be involved in the regulation of cell functions at the pathway level [118]. In the target-pathway networks, the targets located in multiple pathways could be the key targets for complex disease treatment. In addition, it was recently reported that a comprehensive genetic analysis of 24 pancreatic cancers, 63 genetic alterations are identified involved in 12 cellular signaling pathways and processes that were each genetically altered in 67 to 100% of the tumors [119]. Consequently, targeting the physiologic effects of the altered pathways and processes rather than their individual gene components is the promising therapeutic development.

In addition, network analysis shows that relevant signaling pathways are usually safeguarded by well-tuned mechanisms of redundancy. This strengthens the view that the weak but simultaneous modulation of multiple targets is a more promising strategy for triggering a physiological response than the inhibition of a single protein [120]. A theoretical algorithm, such as CIPHER, was proposed to identify the pathways and indicate the network-based computational framework [121-123]. Especially, the molecular and genetic complexity of advanced-stage diseases such as cancer suggests that targeting a single oncogenic pathway may not be sufficient to achieve durable remissions in patients [124]. Accordingly, novel drug discovery and development strategies are focusing on targeting multiple signaling pathways, either with drug combinations or through the design and development of a single compound able to target multiple targets.

5. RATIONAL DESIGN OF MULTI-TARGET DRUGS

The traditional drug discovery process is complex, time consuming and expensive, so rationally designing drugs with a desired multi-target profile is becoming increasingly important [125]. At present, it is a challenging task for the consideration that structure-activity relationships of molecules acts on different biological targets. Some scientists found that ligands where polypharmacology has been deliberately designed in, by conjugation or overlapping pharmacophores, are likely to have lower ligand efficiency than general preclinical compounds [7]. In fact, a multi-target drug is likely to act on several targets with lower affinity than a single-target drug, because it is unlikely that a small, drug-like molecule will bind to a variety of different targets with equally high affinity. However, low-affinity drug binding is apparently not a disadvantage [101]. For example, memantine (30) (a drug used to treat Alzheimer's disease) and other multi-target non-competitive NMDA receptor antagonists show that low-affinity, multi-target drugs might have a lower prevalence and a reduced range of side-effects than high-affinity, single-target drugs [42, 43]. Thus, many multi-target drugs interact with proteins by low-affinity physical interactions, weak, which might be efficient to treat complex diseases.

To identify potential compounds with optimal polypharmacological profiles, a lead compound with the desired biological activity against multiple targets is needed. Moreover, this lead compound needs to be optimized into a clinical candidate that combines the desired polypharmacological profile with a safe, drug-like pharmaceutical profile [7]. Usually, ligands designed by conjugating two distinct pharmacophores are more likely to have high molecular weight and less likely to have oral drug-like physicochemical properties. While for some diseases, the absence of interactions with non-therapeutic off-targets activities contribute to the overall efficacy of a drug [126]. Therefore, it is desirable to assess off-target activities earlier in the drug discovery process. To solve the problem, Milletti and Vulpetti proposed a new method to predict the inhibition map of a compound by comparison of binding pockets. They demonstrated that striking structural similarities at the subpocket level (root mean square deviation (RMSD) < 0.5 Å) may occur among targets with different folds, which can be exploited not only to predict off-target effects but also to design novel inhibitors for targets of interest [127].

Polypharmacology does not preclude the identification of individual targets but focus on the drugs with multiple targets in a disease network that could be modulated to achieve a beneficial clinical outcome. Nowadays, many experimental projects integrated understanding of the interactions among the genome, the proteome, the environment and pathophenome, provide useful insights into the polypharmacological drugs. For example, Apsel *et al.* reported that the systematic discovery of molecules potently inhibit both tyrosine kinases and phosphatidylinositol-3-OH kinases, two protein families that are among the most popular cancer drug targets. Finally, a promiscuous drug-PP121 blocking the proliferation of tumor cells by direct inhibition of both tyrosine kinases and phosphatidylinositol-3-OH kinases was identified [8].

Since experimental testing of molecules on *in vitro* binding assays for thousands of proteins is currently unfeasible, various *in silico* methods have been developed for predicting the pharmacological profile of potential drugs [128]. For example, *in silico* methods based on ligand similarity have been proven very useful in predicting novel targets for known drugs [13, 27 , 47, 129-134]. Virtual screening (pharmacophore-based) and computational docking (biostructure-based) methods were performed to discover poly-pharmacological agents [27]. Docking method is a promising alternative to organize binding sites based on their similarities and assume that they will bind to similar ligands, it is enable to guide discovery of a drug for a disease, because of modeling enables design [135, 136]. And, fragment-based approaches have been proposed to design drugs of multiple targets [137]. The advantage of the method was leaving room for optimization of hit compounds and associated increases in molecular weight, which tended to identify highly efficient small molecules. Structure-based approaches are used to detect and compare binding sites in a computationally more efficient manner, which have emerged as promising new tools to identify phylogenetically targets to which small molecules may show polypharmacology [138, 139]. For example, docking method is a promising alternative to guide the drug discovery, which is to organize binding sites based on their similarities [135, 136]. Searching compounds with multiple targets vastly decreases the drug resistance and toxicity via lowering effective dose [101, 140], thus facilitating discovery of multi-target for complex diseases. The advantages of these computational methods are their applicability to situations in which mechanistic information is incomplete or fragmentary, which will bear the possibility of vastly reducing barriers to drug development. Therefore, computational approaches have been widely extended to multi-target drug discovery campaigns.

Other computational methods were proposed for drug targeting, including statistic and machine learning algorithms. For example, Dar *et al.* developed *Drosophila* model of multiple endocrine neoplasia type 2 combined kinase-focused chemistry, kinome-wide profiling and *Drosophila* genetics to uncover a spectrum of targets contributing to drug-induced efficacy and toxicity, which providing a powerful systems pharmacology approach to develop compounds with a maximal therapeutic index [126]. Furthermore, the team compared the phenotypic effects of the top hit, resulting in 25% of the flies surviving to adulthood, with other library members that had a similar potency against RET (rearranged during transfection) but a distinct activity profile against other kinases. Based on this, they designed a new inhibitor that rescued about 80% of the multiple endocrine neoplasia type 2 (MEN2) flies and was effective to treat thyroid cancer resulting from RET mutations that cause MEN2. Singh *et al.* identified cell type-specific kinases that regu-

late cell migration by elastic net regularization method combined with mRNA expression profiling and previously characterized data on a large set of kinase inhibitors. Broadly, the approach is also generally appropriate for other classes of enzyme inhibitors such as deacetylases and methyltransferases, for which informative target profiles can be obtained easily. These approaches combine aspects of target- and phenotype-based drug discovery, they are thus attractive options for the rational design of multi-target compounds, especially for identifying an optimal polypharmacological profile for treating complex diseases clinically. The goal of drug optimization is to increase activity (efficacy) and decrease toxicity (specificity), thereby improving the therapeutic effect. The computational drug discovery techniques are quite robust, every screening paradigm is prone to errors, therefore, the combination of multiple screening methods is bound to increase the number of false positives significantly, which will lead a new era of effective selective drugs [29]. The future success of novel drug-design paradigm-polypharmacology will depend not only on a new generation of computer models to identify the potential multiple targets, effective drug candidates but also on more-efficient *in vivo* testing.

6. CONLUSION

Polypharmacology is emerging as a novel paradigm in drug discovery, which can be exploited for designing drugs which can effectively target one or more disease states [141]. The FDA approved drugs with a beneficial therapeutic effect mostly target on different target classes and indications, which provides strong support for the concepts of polypharmacology and multi-target compound design. Many complex diseases do not succumb to single-target therapies but rather require a multiple modulation of a network of targets [28]. In recent years, the "one disease-one target" strategy was not successful in the pharmaceutical industry [142], and the network pharmacology have been advocated as the "next paradigm in drug discovery" [7], which offers the promise of tackling the two major sources of attrition in drug development: efficacy and toxicity. However, there remain challenges of how to rationally design ligands with a desired polypharmacological profile, especially across different target classes. In fact, redundant mechanisms can activate multiple pathways, thus impairing the drug efficacy achieved by modulating a single protein activity. In contrast, the multi-target drugs have the potential of improving therapeutic efficacy and safety contribute to the treatment of complex diseases. The superior efficacy of multi-target drugs could also be achieved as a result of their preventing unwanted compensatory mechanisms, which might result in cellular redundancy. In this review, we have highlighted that the features of polypharmacology. Also, we have presented some computational methods to predict the potential multi-target drugs in a range of applications, from the repurposing of existing drugs hit on new protein targets, to designing novel drugs with lower toxicity and higher efficacy. Hence, multi-target drug candidates should be designed by optimizing activity profiles toward the desired targets while minimizing the risk of off-targets. Moreover, network analysis might play important role in repositioning drugs that modulate targets involved in different pathologies. We conclude by noting that, polypharmacology scenario, being able to monitor progress on the binding efficiency of ligands across different target classes associated with therapeutic relevance will contribute to the success of efficacious drugs. The rational polyparmacology design will expand and standard the compound profile and provide new insight in the drug development.

CONFLICT OF INTEREST

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